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Editorial

microRNAs – key players in host-parasite interactions

C. Britton

Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK

Correspondence: Collette Britton, Institute of Biodiversity, Animal Health and Comparative Medicine, Henry Wellcome Building, University of Glasgow Veterinary School, Bearsden Road, Glasgow G61 1QH, UK (e-mail: Collette.Britton@glasgow.ac.uk)

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The discovery of the RNA interference (RNAi) gene-silencing pathway in 1998 revolutionized analysis of gene function (1). In a similar way, the identification of another class of small RNAs, microRNAs (miRNAs), has significantly impacted our understanding of how genes are

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regulated in response to developmental, metabolic and environmental changes. The first miRNA, lin-4, was identified in the free-living nematode *Caenorhabditis elegans* in 1993 (2), but it was not until the discovery of the highly conserved let-7 miRNA in *C. elegans* and higher organisms that the importance of these small RNAs in co-ordinating gene expression in a wide range of species was revealed (3). miRNAs are encoded predominantly in intergenic regions of the genome and are transcribed firstly as hairpin precursor sequences which are cleaved by the endonucleases Drosha and Dicer to form the mature double-stranded ~22 nucleotide miRNA. One strand of the mature miRNA is incorporated into the RNA-induced silencing complex (RISC) and through partial sequence complementarity, most commonly within the 3'UTR, directs the RISC to target mRNAs. This binding leads to translational inhibition and target mRNA degradation (4). miRNAs are therefore key negative regulators of mRNA and protein levels. They can target multiple genes often functioning in the same pathway, adding robustness to cellular responses and enabling them to act as switches of gene expression. These characteristics of miRNAs add an additional level of control to many physiological networks, including the immune response.

The availability of genome sequence data for an increasing number of parasitic organisms and/or their hosts has paved the way to identify the roles of miRNAs during parasite infections. This Special Issue brings together current knowledge of some of the effects of miRNAs on parasite survival, with an emphasis on host-parasite interactions. Some parasites express miRNAs that are released into the host tissue and are speculated to influence immune outcome. On the other hand, the expression profile of host miRNAs is often altered during parasite infections and can impact on susceptibility and resistance, as well as on the pathology of infection. The regulatory effects of parasite miRNAs on the host and vice versa have likely played an important role in the evolution of parasites and their host species and may influence

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parasite host specificity and tropism. Indeed, it is proposed that viruses, such as hepatitis C and equine encephalitis virus (5,6), as well as the protozoan parasite *Theileria* (7) exploit host miRNAs for their own replication and survival.

EXTRACELLULAR VESICLES AS TRANSPORTERS OF PARASITE miRNAs

A number of studies have shown that parasites, especially helminths, can release miRNAs into the host environment, although exactly how these are released and what effects they have on host cells are areas of current investigation. Two papers in this issue, Fromm et al. (8) and Quintana et al. (9), summarise data on extracellular vesicles (EVs) as transporters of miRNAs, as well as other molecules, from flukes and nematodes to host cells. EVs are lipid-enclosed microvesicles that are currently a major area of research due to their roles in cell-cell communication not only in parasite infections but also in other diseases, including cancer (10). Release of EVs from helminth parasites was initially demonstrated from the trematodes *Echinostoma caproni* and *Fasciola hepatica* (11) and subsequently shown for nematodes (12) and schistosomes (13). EVs contain proteins and parasite-derived miRNAs that are speculated to play key roles in interspecies communication and host immune modulation. Fromm et al. (8) summarise current knowledge of the miRNA repertoire of *F. hepatica*, focussing on sequence data from small RNA libraries prepared from juvenile and adult fluke and from adult EVs. This identified stage-specific differences in miRNA profile and enrichment of some miRNAs in EVs, indicating selective packaging. Stage-specific differences in the RNA cargo of EVs from the filarial nematode *Brugia malayi,* are also discussed by Quintana et al. (9). Elucidating the mechanisms controlling the production of EVs from different parasite stages and their site of origin is important in progressing our understanding of parasite immunomodulation and revealing potential novel targets for intervention. An interesting

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feature to emerge from sequencing of miRNAs released from parasitic helminths, either in EVs or in excretory-secretory products, is the abundance of sequences orthologous to vertebrate miRNAs (12,14). Whether these regulate the same target genes as host miRNAs will require further investigation. In addition, as discussed by Quintana et al. (9), the ability to detect released miRNAs in host serum presents an opportunity to develop improved diagnostic markers of infection.

miRNAs REGULATE MUCOSAL IMMUNE RESPONSES

Host immune responses to infection with helminth and protozoan parasites are highly complex and not surprisingly, miRNAs play essential roles in co-ordinating and fine-tuning many aspects of the mammalian immune response (15,16). Details of miRNA-mediated regulation of immune responses to gastrointestinal (GI) nematodes (17) and to *Cryptosporidium* (18) are reviewed in this issue. Immunity and expulsion of GI nematodes involves a range of cell types and cytokines. The importance of miRNAs in regulating these responses is demonstrated by genetic deletion of the miRNA processing enzyme Dicer or of specific miRNA loci, as summarized by Entwistle and Wilson (17). Loss of Dicer alters the intestinal architecture and results in increased susceptibility to infection with the GI nematode *Trichuris muris.* A number of individual miRNAs and miRNA clusters have been identified in promoting specific T helper (Th) cell lineages and functions, and of particular note are the roles of miR-155 in protective T and B cell responses to GI nematodes. While altering miRNA levels is likely to have many effects on immune homeostasis, Entwistle and Wilson (17) suggest that transient manipulation of specific miRNAs could be exploited to induce protective immune mechanisms during vaccination.

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Infection of gut epithelium with the protozoan parasite *Cryptosporidium parvum* results in activation of the nuclear factor kappa B (NF-kB) signaling pathway. As reviewed by Ming et al. (18), this leads to changes in the expression profile of host miRNAs. While some of these may be general anti-microbial responses, others are specific to *C. parvum* infection. It is proposed that these miRNAs provide both positive and negative feedback regulation to key signaling pathways, including enhanced Toll-like receptor expression but reduced inflammatory signaling, to fine-tune the immune response. Interestingly, infection with *C. parvum* leads to release of exosomes, a type of extracellular vesicle, from the GI epithelium. Whether these exosomes contain miRNAs that may modulate adaptive immunity via epithelial-immune cell communication is not yet known, but further analysis of host miRNA function, particularly using in vivo studies, will be important for understanding mucosal immunity.

miRNA REGULATION MAY CONTRIBUTE TO IMMUNOPATHOLOGY OF PARASITE INFECTIONS

The intracellular protozoan parasite *Toxoplasma gondii* has been used extensively as a model for studying host-parasite interactions and indeed this system was the first to demonstrate changes in host miRNA profile in response to parasite infection (19). Cai and Shen (20) review in vitro and in vivo studies using miRNA knockout mouse strains that have demonstrated the importance of the pro- and anti-inflammatory regulators mir-155 and mir-146a, respectively, in influencing the pathology of infection. In addition, induction of specific miRNAs following infection may contribute to the neuropathology of *T. gondii* infection by altering neuronal cell regulation and function. Identifying changes in miRNA profile in response to parasite infections and the networks these miRNAs regulate is highly informative

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in explaining immunopathology and may lead to novel therapies to reduce these effects. Mir-155 and mir-146 were also among the miRNAs upregulated in the liver following infection with the human-infective trematode *Schistosoma japonicum* and, as reviewed by Hong et al. (21), may regulate the inflammatory and T and B cell responses to schistosome egg antigens. Several miRNAs have been associated with regulation of hepatic fibrosis and future studies aimed at testing the effects of mimics of these will be important in determining their potential therapeutic value in reducing liver pathology.

miRNAs IN PARASITE VECTORS

In addition to the attention given to miRNAs in influencing immune outcome in the vertebrate host, there is increasing interest in the roles of miRNAs within vector hosts in influencing parasite development and survival. In *Anopheles* mosquitoes, which transmit *Plasmodium,* knowledge of miRNAs is in its infancy but already reveals interesting findings with potential applications to vector and parasite control, as reviewed by Lampe and Levashina (22). Changes in miRNA expression profile in mid-gut and ovary tissue have been identified following a blood-meal and roles in development, reproduction and immunity identified. Interestingly, infection with *P. falciparum* resulted in increased expression of Dicer and Drosha, suggesting an increase in miRNA biosynthesis in response to infection, although silencing of these genes had no effect on parasite numbers. The technology to deliver miRNA mimics and inhibitors and the feasibility of CRISPR/Cas9 deletion of specific miRNAs in insect vectors should allow identification of miRNAs essential for parasite and/or vector survival.

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There is also a pressing need for reliable molecular tools to knockout or over-express parasite miRNA genes, particularly for helminth parasites, to determine the functions of specific miRNAs in development and host-parasite communication. In addition, availability of a greater range of miRNA-knockout mice will advance our understanding of the importance of host miRNAs in influencing immunity and pathology. Such studies will provide a detailed picture of the mechanisms and networks influencing parasite development and survival within the host and should lead to new targets for therapeutic intervention.

Finally, I would like to thank all the authors who contributed to this Special Issue by providing a very comprehensive and up to date review of the importance of miRNAs in host-parasite interactions, and to the reviewers who generously gave their time to provide advice on the final articles.

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